

## Characterization and Presumptive Identification of *Helicobacter pylori* Isolates from Rhesus Monkeys

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We characterized 38 *Helicobacter* isolates, including 22 from gastric biopsy samples obtained from 14 rhesus monkeys and single isolates from 16 monkeys in a different colony. Biochemical profiles of these isolates were nearly identical to that of *Helicobacter pylori* ATCC 43504. Restriction fragment length polymorphism (RFLP) analysis indicated that each infected monkey harbored one to four strains. The 17 RFLP types found among these 22 isolates differed from all seven RFLPs found among the other 16 isolates. Thus, monkeys within a given colony are more likely to be infected by *Helicobacter* isolates with the same or a similar RFLP than are monkeys from different colonies. A 16S rRNA gene was amplified by PCR and cloned from the *Helicobacter* isolate from rhesus monkey 85D08. Ribotyping with this probe demonstrated less diversity among isolates from rhesus monkeys than was reported among isolates of *H. pylori* from humans, as did RFLP analysis of a PCR fragment of the *ureA-ureB* gene cluster. The DNA sequence of the cloned 16S rRNA gene was determined and compared with sequences reported for *H. pylori* and other *Helicobacter* species. Our analysis of 127 nucleotides (corresponding with residues 1240 to 1366 of the *Escherichia coli* 16S rRNA gene) indicated that the *Helicobacter* isolate from monkey 85D08 was 99.2 to 100% homologous to isolates of *H. pylori* from humans but only 83.5 to 96.9% homologous with other *Helicobacter* species in this region of the 16S rRNA gene. These data provide strong support for the presumptive identification of these isolates as *H. pylori*.

*Helicobacter pylori* is a gram-negative spiral bacterium found in the gastric mucosa of the vast majority of patients with gastritis and peptic ulcer disease, but it is also found in a large number of asymptomatic individuals (1). In addition, there is a greater frequency of chronic gastric colonization by *H. pylori* in patients with gastric adenocarcinoma than in matched controls (24). Putative virulence factors of *H. pylori* include production of urease, catalase, and cytotoxin(s), as well as motility and the ability to adhere to gastric mucosa, but the pathogenic mechanisms of *H. pylori* infection are not yet well defined (1, 6). Natural infections with *Helicobacter* spp. have been found in a variety of animals including nonhuman primates (2, 9, 15). Several animal species have been tested as experimental models for *H. pylori* infection, but no system mimicking *H. pylori* pathogenesis in humans has yet been completely successful (7, 9, 11).

Natural infection of rhesus monkeys by bacteria that resemble *H. pylori* is associated with chronic gastritis similar to that seen in humans infected by *H. pylori* (4, 5). These bacteria are antigenically, immunologically, and morphologically indistinguishable from human strains of *H. pylori* (5, 15). Furthermore, Ho et al. (10) demonstrated complete nucleotide sequence homology, over a 275-nucleotide segment of 16S rRNA genes isolated by amplification using PCR, between two isolates from rhesus monkeys and a clinical *H. pylori* strain. Therefore, the rhesus monkey is a potentially useful model for *H. pylori* infection in humans. However, the molecular epizootiology in monkeys is unknown. Restriction fragment length polymor-

phism (RFLP) analysis and ribotyping have been used to examine the molecular epidemiology of *H. pylori* infection in humans. In the present study, we cultured and characterized spiral bacteria from gastric biopsy samples of rhesus monkeys housed in one colony. Some of the monkeys were also used in a previously reported study (4). We extended the study to include *Helicobacter* isolates from rhesus monkeys in another colony. We examined the relationship among these isolates, *H. pylori* obtained from humans, and other *Helicobacter* species.

### MATERIALS AND METHODS

**Microbiology.** All experiments were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* (28). Videogastrosopies were performed to harvest gastric mucosal biopsy samples from the corpora and antra of monkeys housed in colony A (5). Two biopsy samples from each region were placed in 100  $\mu$ l of 0.9% saline and kept on ice until processed. Biopsy samples were ground with a sterile glass pestle, and 30  $\mu$ l was inoculated onto Campy agar (Remel, Lenexa, Kans.) and incubated in 7.5% O<sub>2</sub>–7.5% CO<sub>2</sub>–85% N<sub>2</sub> at 37°C for 5 to 7 days. Several isolated colonies (0.5 mm in diameter, colorless, and convex) resembling *H. pylori* were examined for urease activity (27) and subcultured on sheep blood agar. After 5 to 7 days, there was sufficient growth for passage and analysis of these isolates. Additional biopsy samples were examined by histology. *Helicobacter* strains numbered 30986 to 30997, 30999 to 31001, and 31008, isolated from infected rhesus monkeys housed at another facility (kindly provided by G. Zurenko, Upjohn Company, Kalamazoo, Mich.), were designated as isolates from monkey colony B. Human *H. pylori* ATCC 43504 was purchased from the American Type Culture Collection (Rockville, Md.). Isolates were characterized by the rapid urease test (27), oxidase

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test (Becton Dickinson, Cockeysville, Md.), catalase test (formation of bubbles in 3% H<sub>2</sub>O<sub>2</sub>), and Gram stain. Biochemical profiles were examined using the An-IDENT system (Analytab Products, Plainview, N.Y.). Isolates were stored at -70°C in brucella broth containing 10% glycerol. The *Helicobacter* isolate from rhesus monkey 85D08 was deposited with the American Type Culture Collection and designated ATCC 51407.

**Preparation of chromosomal DNA.** Multiple colonies of each *Helicobacter* isolate were harvested from sheep blood agar with a cotton swab, suspended in 30 to 100 ml of brucella broth containing 10% fetal bovine serum, and incubated microaerobically, as described above or with the CampyPak Plus system (BBL Microbiology Systems, Cockeysville, Md.), at 75 rpm and 37°C for 4 days. DNA was prepared (17, 21) with ethidium bromide-high-salt extraction (23) and resuspended in TE (20).

**Cloning and sequencing of a 16S rRNA gene from a *Helicobacter* strain isolated from a rhesus monkey.** Universal primers 8FPL and 1492RPL, designed to amplify 16S rRNA genes from all eubacteria (22), were kindly provided by J. V. Solnick (Stanford University, Stanford, Calif.). Reaction mixtures (100 µl) in standard PCR buffer (Perkin-Elmer Cetus, Norwalk, Conn.), containing each of the four deoxyribonucleotides at 50 µM, 25 pmol of each primer, and 50 ng of chromosomal DNA from the *Helicobacter* isolate from monkey 85D08, were overlaid with mineral oil and denatured at 95°C for 5 min. AmpliTaq (2.5 U in 5.0 µl of 1× buffer) was added to each reaction mixture without removing the tube from the heat block, and PCR was performed at 94°C for 1 min, 61°C for 1 min, and 72°C for 2 min for 35 cycles, followed by 10 min at 72°C.

The 1.5-kb PCR product was desalted with 400 µl of sterile water, concentrated with an Ultrafree-MC filter unit (Millipore Products Division, Bedford, Mass.), and digested to completion with *NotI* at 37°C for 1 h. The DNA was extracted with ethidium bromide-high salt (23), and 1 µl (20 µg) of glycogen was added (Boehringer-Mannheim Corp., Indianapolis, Ind.). The DNA was precipitated, washed, dried, and resuspended in water. Plasmid pBluescript II KS+ (Stratagene Cloning Systems, La Jolla, Calif.) was digested with *NotI*, dephosphorylated with calf intestinal alkaline phosphatase (Boehringer-Mannheim Corp.), and purified as described above. *Escherichia coli* DH5αMCR (GIBCO-BRL, Gaithersburg, Md.) was transformed with the ligated fragments (insert/vector ratio, 2:1; 16°C, 16 h) (20).

Plasmid p16S1-5, selected by a plasmid screen of ampicillin-resistant transformants, contained a 1.5-kb *NotI* insert. PCR amplification with the 16S rRNA universal primers 8FPL and 1492RPL and the HP1 and HP3 primers, which amplify a 446-bp fragment of the 16S rRNA gene from *H. pylori* but not *Helicobacter mustelae* or *Helicobacter cinaedi* (10), indicated that p16S1-5 contained a 16S rRNA gene very closely related to the *H. pylori* gene. The sequences of both DNA strands of the cloned fragment were determined by double-stranded sequencing (Sequenase version 2.0; United States Biochemical, Cleveland, Ohio). Sequencing primers included 8FPL, 1492RPL, vector primers KS and T7, and newly synthesized primers based on p16S1-5 derived sequences.

The sequence of the 16S rRNA gene and the immediately contiguous 5' and 3' chromosomal DNA regions was completed by thermal cycle sequencing (CircumVent; New England BioLabs, Beverly, Mass.). Two oligonucleotides (20F, 5'-TTGCCTTAAGTCAGGATGC3', and 21R, 5'-CAAGCTA GAAGCTTCATCG3'), synthesized on the basis of sequences located approximately 60 nucleotides from either end of the

cloned fragment, were end labeled with [ $\gamma$ -<sup>32</sup>P]dATP and T4 polynucleotide kinase. Reaction mixtures containing 2 µg of chromosomal DNA from the *Helicobacter* 85D08 isolate, 2.5 pmol of a single primer, deoxy and dideoxy sequencing mixes, and buffer were amplified for 20 s each at 95, 54, and 72°C for 25 cycles. The sequencing reaction products were separated by polyacrylamide gel electrophoresis, fixed, dried, and exposed to film with intensifying screens at -70°C for 24 to 72 h.

**DNA sequence analysis.** DNA sequences were compared by using the GCG Sequence Analysis Software Package (3). DNA sequences for human *H. pylori* and other *Helicobacter* species were obtained from GenBank or reference 19 (see Fig. 1). Unresolved nucleotides were omitted when the percent sequence homology between two strains was determined. Single nucleotide alignment gaps were assigned half the value of a nucleotide in the same position (19). DNA homology was calculated as follows: % homology = [(number of bases - number of base changes)/number of bases] × 100. The sequence alignments of two regions corresponding with nucleotides 711 to 873 and 1240 to 1366 of the *E. coli* 16S rRNA gene, for which several human *H. pylori* sequences are available, were examined further.

**Restriction endonuclease digestion and gel electrophoresis.** Approximately 3 µg of chromosomal DNA was digested with excess *HaeIII* for 2 h (GIBCO-BRL). The fragments were separated by electrophoresis in 0.8% agarose gels in TAE buffer (20) for 16 h at 18 V. Gels were stained with ethidium bromide and destained in water prior to photography (20).

**Ribotyping of *Helicobacter* isolates from rhesus monkeys.** Electrophoresed chromosomal digests were transferred to nylon membranes (Nytran; Schleicher & Schuell, Keene, N.H.) (20) by positive pressure and cross-linked with UV light (Posiblot and Stratalinker; Stratagene Cloning Systems).

The 1.5-kb *NotI* fragment containing the 16S rRNA gene was excised after electrophoresis through 0.75% low-melting-point NuSieve GTG agarose (FMC BioProducts, Rockland, Maine) in TAE buffer and melted with water to a final DNA concentration of 2.5 ng/µl. The DNA probe was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by random priming (Prime-It II; Stratagene Cloning Systems) and purified on a G-50 Sephadex Quick Spin column (Boehringer-Mannheim Corp.).

The blots were prehybridized for 2 h, hybridized overnight with labeled probe (25 ng), washed as described by the manufacturer (Schleicher & Schuell), and exposed to Kodak X-Omat AR5 film at -70°C with intensifying screens for various times.

**Additional characterization of *Helicobacter* isolates from rhesus monkeys.** Selected bacterial isolates were swabbed off sheep blood agar, washed, and prepared for pulsed-field gel electrophoresis of intact chromosomal DNA by following the manufacturer's instructions (FMC BioProducts). Processed plugs were electrophoresed in 1% SeaKem GTG gel in 0.5× TBE buffer (20) at 200 V with a 0.9- to 100-s ramp for 20 h at 12°C with a clamped homogeneous electric field electrophoresis cell (Bio-Rad, Hercules, Calif.).

Chromosomal DNA from selected isolates was amplified with primers that produce a 2.4-kb fragment of the *ureA-ureB* gene cluster (8) or with 8FPL and 1492RPL, producing a 1.5-kb fragment of the 16S rRNA gene. The PCR products were desalted and concentrated as described above, digested with a variety of restriction endonucleases, and run on 4% NuSieve GTG agarose gels in TAE buffer (20).

**Nucleotide sequence accession number.** The sequence for the cloned 16S rRNA gene of the *Helicobacter* isolate from monkey 85D08 is available from GenBank under accession number U00679.

<i>H. pylori</i> 85D08 <sup>a</sup>	TGGGGTGCAC AAAGAGAAGC AATACTGCGA AGTGGAGCCA ATCTTCAAAA CACCTCTCAG TTCGGATTGT	
<i>H. pylori</i> 5443 <sup>b</sup>	.....	
<i>H. pylori</i> NTCT 11638 <sup>c</sup>	.....	
<i>H. pylori</i> NTCT 11637 <sup>c</sup>	.....	A..
<i>H. acinonyx</i> ATCC 29263 <sup>c</sup>	..... G.....	.....C..
<i>H. felis</i> ATCC 49179 <sup>c</sup>	.....T... ..G.C.... G..T.....	.....A.... .NN.....C
<i>H. felis</i> DS3 <sup>c</sup>	.....T... ..T... ..G.C.... G..CT.....	.....A.... .T.....C
<i>H. fennelliae</i> CCUG 18820 <sup>c</sup>	..... ..TC.... GA.....A....	.....C.... .G.....
<i>H. sp.</i> CCUG 29259 <sup>c</sup>	.....A.... ..C.... ..C.... G.....A....	.....CA.... .T.....
<i>H. mustelae</i> ATCC 43772 <sup>c</sup>	..... ..C.... ..C.... G.....A....	.....CA.... .T.....
<i>H. sp.</i> 91-169-21 <sup>c</sup>	.....TA..T... ..C.... G.....A....	.....CA.... .TA.....
<i>H. muridarum</i> ATCC 49282 <sup>c</sup>	..... ..G.... ..TC..T... G.....A....	.....CT.... .GT.....
<i>H. cinaedi</i> CCUG 18818 <sup>c</sup>	.....ACAT.. ..A..T... ..TC.... GA.....A....	.....CT.... TGT.....
<i>H. pylori</i> 85D08	AGGCTGCAAC TCGCCTGCAT GAAGCTGGAA TCGCTAGTAA TCGCAAATCA GCCATGT	% Homology
<i>H. pylori</i> 5443	.....N.. ..N.....	100.0
<i>H. pylori</i> NTCT 11638	..... ..N.....	100.0
<i>H. pylori</i> NTCT 11637	..... ..N.....	99.2
<i>H. acinonyx</i> ATCC 29263	..... ..C.....	96.9
<i>H. felis</i> ATCC 49179	..... ..N.....	94.4
<i>H. felis</i> DS3	..... ..N.....	92.1
<i>H. fennelliae</i> CCUG 18820	..T..... ..A..... A.....	91.3
<i>H. sp.</i> CCUG 29259	..T..... ..A..... ..N.....	90.5
<i>H. mustelae</i> ATCC 43772	..T..... ..A..A....	90.5
<i>H. sp.</i> 91-169-21	..T..... ..A..A....	89.8
<i>H. muridarum</i> ATCC 49282	..T..... ..A.....	88.2
<i>H. cinaedi</i> CCUG 18818	..T..... ..A..A.... A.....	83.5

FIG. 1. Alignment of a partial 16S rRNA gene sequence of *H. pylori* isolated from rhesus monkey 85D08 with previously published sequences from three *H. pylori* strains isolated from humans and other *Helicobacter* spp. (5'-to-3' sequence corresponds with the *E. coli* 16S rRNA gene sequence from position 1240 to 1366 [127 nucleotides]). Dots indicate bases identical to those at corresponding positions in the rhesus monkey 85D08 sequence. G, guanine; A, adenine; T, thymine; C, cytosine; N, unidentified bases. Sequences were obtained from this study (a), reference 19 (b), and GenBank (c). Percent homology is relative to the *H. pylori* 85D08 sequence.

## RESULTS

**Characterization of *Helicobacter* isolates.** Of the 23 rhesus monkeys from colony A, examined by endoscopy and culture of gastric biopsy samples, 14 (60.9%) were infected with gram-negative, curved, rod-shaped bacteria that expressed oxidase, catalase, and strong urease activities. Positive cultures correlated on a one-to-one basis with histopathological evidence of gastritis in hematoxylin and eosin-stained biopsy sections. Five independent *H. pylori*-like colonies were selected from each primary culture on Campy agar. The variation in the number of isolates presumptively identified as *H. pylori*, 1 to 11 per monkey, reflected the fact that biopsies were obtained (i) from either the corpus or the antrum or both sites and (ii) from multiple endoscopies for some of the animals. RFLP analysis of *HaeIII* digests of chromosomal DNA from these isolates, which provides high-resolution differentiation, indicated that each infected monkey carried one to four different strains.

Phenotypic profiles of *Helicobacter* isolates were determined by using An-IDENT strips plus tests for urease, catalase, and oxidase activities and Gram stains. The strains tested included 22 isolates representing each RFLP type from colony A monkeys, 16 isolates from colony B monkeys, and the *H. pylori* reference strain ATCC 43504. Preformed enzyme activities of these isolates were indistinguishable from activities of *H. pylori* 43504 enzymes for all tests with the exception of leucine aminopeptidase. *H. pylori* 43504 and 58% of the monkey isolates were positive for this enzyme, whereas the remainder had intermediate activities. Isolates from monkeys in colonies A and B were distributed similarly between the groups with positive (59 and 56%, respectively) and intermediate (41 and 44%, respectively) activities. All isolates were positive for phosphatase, catalase, oxidase, and urease activities and leucine, arginine, and alanine aminopeptidase activities.

Preliminary attempts to demonstrate differences among the

*Helicobacter* strains isolated from rhesus monkeys were made by testing for RFLP of PCR-amplified products. A 2.4-kb fragment of the *ureA-ureB* gene cluster was digested with *HaeIII*, *DdeI*, *HhaI*, and *MspI* restriction endonucleases, but polymorphism was observed only with *MspI* fragments and was very limited (data not shown). Similarly, RFLP testing of an *AluI* digest of the 1.5-kb PCR product of the 16S rRNA gene revealed identical patterns of restriction fragments in eight of nine isolates examined (data not shown). These results indicated that genetic variation of the *ureA-ureB* and 16S rRNA genes examined by RFLP testing was not particularly useful for differentiation of *Helicobacter* isolates from rhesus monkeys.

**DNA sequence analysis of *Helicobacter* 16S rRNA genes.** We analyzed the DNA sequence alignment of the 16S rRNA gene, cloned from the *Helicobacter* isolate from monkey 85D08, with partial 16S rRNA gene sequences available for *H. pylori* strains from humans and for other *Helicobacter* spp. We did not find the sequence corresponding with nucleotides 711 to 873 of the *E. coli* 16S rRNA gene useful for distinguishing between species within the genus *Helicobacter*, in contrast to the suggestion by Ho et al. (10). DNA homology in this region ranged from 94.2 to 99.4% among human isolates, from 95.9 to 99.1% between strain 85D08 and the four human *H. pylori* strains, and from 92.4 to 99.1% between strain 85D08 and the other *Helicobacter* spp. In contrast, comparison of homologies within the 127-nucleotide region corresponding with positions 1240 to 1366 in the *E. coli* 16S rRNA gene (Fig. 1) demonstrated that strain 85D08 was much more homologous with the available sequences from three *H. pylori* isolates from humans (99.2 to 100% homology) than with any of the other *Helicobacter* spp. (83.5 to 96.9% homology). Among other *Helicobacter* species, *Helicobacter acinonyx* was most similar to strain 85D08 (96.9% homology) in this 16S rRNA gene sequence and *H. cinaedi* was least homologous (83.5%). These results indi-

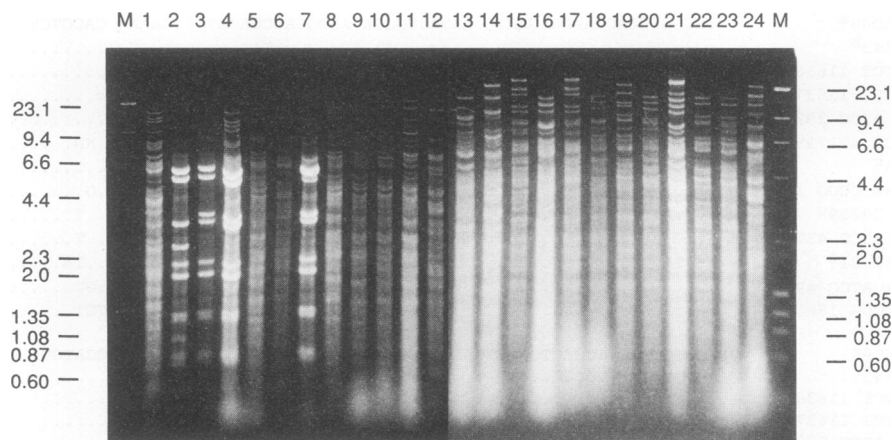


FIG. 2. RFLP analysis of *Hae*III-digested genomic DNA from *H. pylori* isolates from rhesus monkeys from colony A. For the DNA samples listed below, the designations are the monkey identification number and the lowercase letters specify individual isolates from a given monkey. Lanes: 1, 85D08; 2, 7R3-a; 3, 7R3-b; 4, 86D60-a; 5, 86D60-b; 6, 86D60-c; 7, 86D60-d; 8, 86D30-a; 9, 86D30-b; 10, 7MG; 11, 82A49; 12, type strain *H. pylori* ATCC 43504; 13, 8B1-a; 14, 8B1-b; 15, 8PZ; 16, 91R; 17, 8R1-a; 18, 8R1-b; 19, 9A4; 20, 8QX-a; 21, 8QX-b; 22, 8RH; 23, 7N1; 24, type strain *H. pylori* ATCC 43504; M,  $\lambda$  phage, *Hind*III plus  $\phi$ X174, *Hae*III restriction fragments (GIBCO-BRL) as size standards. Pairs of strains with common RFLPs are 7R3-b and 86D60-a, 7MG and 7N1, 8PZ and 8R1-a, and 91R and 8R1-b.

cate that the region of the 16S rRNA gene corresponding with nucleotides 1240 to 1366 in the *E. coli* cognate gene is useful for distinguishing species within the genus *Helicobacter* and demonstrate that the strain from rhesus monkey 85D08 is very closely related to *H. pylori* from humans.

**RFLP testing and ribotyping.** Seventeen RFLP patterns, four of which were found in pairs of animals, were observed with electrophoresed *Hae*III-digested DNA from *Helicobacter* isolates recovered from monkeys in colony A (Fig. 2). Pulsed-field gel electrophoresis detected a 26-kb plasmid (data not shown) in isolates that showed multiple intense DNA bands by RFLP testing with the identical sum total size. Four ribotypes were identified among the 17 different RFLP patterns (Fig. 3). Thirteen isolates had the most common ribotype, seven had an additional 2.1-kb band, and two had unique ribotypes.

*Helicobacter* isolates from colony B monkeys demonstrated seven RFLP patterns (data not shown), none of which was seen in the first set of strains, and three ribotypes (data not shown). Although the most frequent ribotype (Fig. 3, lane 1) was identical in both colonies (14 of 22 from colony A and 11 of 16 from colony B), there was no consistent correlation between the common RFLP pattern and ribotype. *H. pylori* 43504

differed by RFLP pattern and ribotype from all of the *Helicobacter* isolates from monkeys.

## DISCUSSION

Morphological, antigenic, and biochemical analysis of a small number of *Helicobacter* isolates from rhesus monkeys provided evidence that these bacteria were indistinguishable from human isolates of *H. pylori* (5, 15). We confirmed and extended these results by performing more biochemical and molecular epidemiological tests on a larger collection of selected *Helicobacter* isolates from rhesus monkeys. The preformed enzyme profiles of all strains were identical, with the exception of leucine aminopeptidase. We detected this enzyme activity after 4 h in all isolates but at intermediate levels in 42% of the isolates. In contrast, McNulty and Dent (13) reported activity in all 162 human *H. pylori* strains examined after 18 h of incubation, using a different test system (Rosco Diagnostica). The variability we observed in leucine aminopeptidase activity may arise from differences in the substrate or incubation time. Alternatively, *Helicobacter* isolates from rhesus

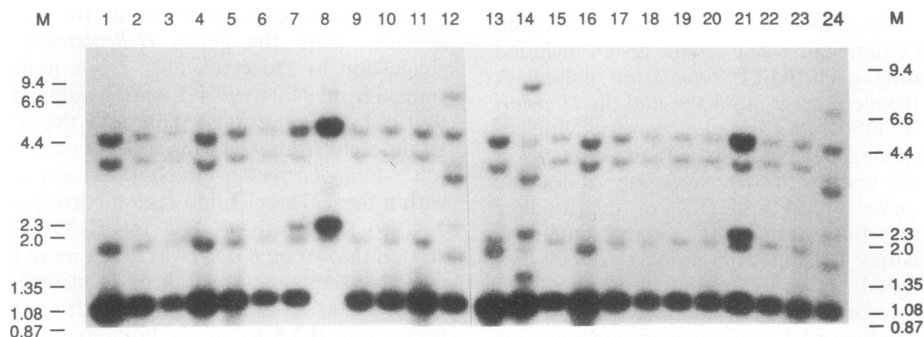


FIG. 3. Ribotyping by hybridization of  $^{32}$ P-labeled 16S rRNA gene from *H. pylori* to *Hae*III-digested DNA from *H. pylori* isolated from rhesus monkeys from colony A. Lane numbers and sample identities are the same as in Fig. 2. Lane 1 represents the most common ribotype. Lane 5 shows the second most common ribotype. Lanes 8 and 14 demonstrate unique ribotypes.

monkeys may demonstrate more innate variability for this enzyme than human *H. pylori*.

RFLP testing and ribotyping are powerful methods for studying the molecular epidemiology of *H. pylori*. Majewski and Goodwin (12) reported major RFLP differences in pairs of consecutive isolates from five patients. In contrast, other investigators reported persistence of a single *H. pylori* strain over time (21, 26). Rauws et al. (18) showed that eight family members were infected with a common strain of *H. pylori*, but other researchers reported that family members were infected with different strains (12, 21). Tee et al. (26) reported that strains from 64 of 100 subjects had unique ribotypes, while strains with the remaining patterns were isolated from two to five subjects. RFLP testing and ribotyping in these earlier studies demonstrated extensive genomic variation among isolates of *H. pylori* from humans.

Molecular probes used for ribotyping *H. pylori* include cDNA prepared from either *E. coli* or *H. pylori* 16S plus 23S rRNA and cloned *E. coli* 5S, 16S, and 23S rRNA and tRNA<sub>2</sub><sup>Glu</sup> genes (14, 16, 26). Taylor et al. (25) showed that only two of three *H. pylori* 16S genes hybridized to a 16S gene cloned from *Campylobacter jejuni* while the third gene was only detected with a 16S gene PCR amplified from *H. pylori*. To maximize detection in the present study, the ribotyping probe was a 16S rRNA gene cloned from a *Helicobacter* isolate from a rhesus monkey. In contrast to the extensive variation detected by ribotyping among human *H. pylori* isolates, the ribotypes of *Helicobacter* from monkeys were much less variable. The high degree of similarity in ribopatterns indicated that the *Helicobacter* strains infecting rhesus monkeys from two different colonies were more closely related than are strains isolated from humans. In the present study, ribotyping was not as powerful as RFLP testing of chromosomal DNA as a method for distinguishing between *Helicobacter* isolates infecting rhesus monkeys.

Recently, Ho et al. (10) found 100% homology when they compared the DNA sequences of 275 bases of a PCR-amplified 16S rRNA gene from one clinical and two rhesus monkey isolates. In contrast, they found only 97.3% homology between their clinical isolate and *H. pylori* NCTC 11638. We constructed DNA sequence alignments of a 16S rRNA gene cloned from a *Helicobacter* strain isolated from monkey 85D08 using all available *Helicobacter* 16S sequences from humans and animals. Our analysis revealed that homology levels over the 166 bases of the region sequenced by Ho et al. varied to a greater extent among human isolates than among the isolates from humans and rhesus monkey 85D08. However, the extent of sequence homology between the isolate from monkey 85D08 and the *Helicobacter* spp. other than *H. pylori* was comparable. These findings indicate that sequence analysis based on the region of the 16S gene from position 711 to 873 of the *E. coli* gene does not provide sufficient discrimination to support the conclusion of Ho et al. (10) that animal gastric isolates are of human origin. In contrast, our analysis of the 127-base sequence, corresponding with nucleotides 1240 to 1366 in the *E. coli* 16S rRNA gene, demonstrated a clear separation between one group containing the *Helicobacter* strain isolated from rhesus monkey 85D08 and *H. pylori* from humans and a second group composed of all other *Helicobacter* spp. examined. Studies of hybridization of genomic DNAs will be needed to demonstrate definitively whether the *Helicobacter* isolates from monkeys belong to the species *H. pylori*. However, the data from our DNA sequence analyses, molecular epidemiologic studies, and biochemical tests presented here provide strong evidence for presumptive identification of the *Helicobacter* isolates from naturally infected rhesus monkeys as

*H. pylori*. Therefore, we believe that the rhesus monkey model is a good system for studying the pathophysiology of *H. pylori* infection.

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